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A Synthetic "Trigeneric" Hybrid, × *Asplenosorus pinnatifidus* × *Phyllitis scolopendrium* var. *americana*

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Hybrids in artificial cultures have contributed to the understanding of the systematic morphology of ferns. Since the classic work of Margaret Slosson (1902), which first proved the origin of the natural hybrid ×*Asplenosorus ebenoides* (R. R. Scott) Wherry (=*Asplenium platyneuron* (L.) B.S.P. × *Camptosorus rhizophyllus* (L.) Link), the techniques of growing fern gametophytes and producing crosses have improved, and many new developments have ensued. In 1957, Wagner and Whitmire provided the first demonstration of the conversion of a sterile allodiploid fern to a fertile allotetraploid. In 1968, Lovis reconstructed a fertile hybrid species of fern (*Asplenium* (×)*adulterinum* Milde). In this article, all taxa, fertile and sterile, of interspecific origin will be referred to as "nothospecies," and indicated by the use of the times sign if sterile and with parentheses around the sign if fertile—i.e., (×), a convention proposed by C. Werth (pers. comm.); divergent species will be referred to as "orthospecies" and will lack the multiplication sign.

Many important experiments on Aspleniaceae were accomplished in European laboratories especially, as discussed and summarized by Reichstein (1981). In almost all cases, such experimental hybridizations were carried out to test some hypothesis of the origin of a given nothospecies. By comparison, many fewer hybridizations have been undertaken simply to find out what a cross between taxon A and taxon B might look like, and what combining, for example, a creeping rhizome with an upright caudex, or hairs with scales, or discrete sori with acrostichoid sori, might yield morphologically. Yet such questions may bear upon our understanding of the determinants of structure and form; we may be able to gain insights that would otherwise be unavailable. The plants involved in this report are all members of the spleenwort family, Aspleniaceae, always popular objects for culture work and hybridization experiments because of their conveniently small size, ease of culture, rapid growth, and often very distinct forms.

As a matter of fact, the bizarre hybrid that we briefly describe below was formed by accident. A terrarium containing numerous gametophytes of the lobed spleenwort, (×)*Asplenosorus pinnatifidus* (Muhl.) Mickel (=mountain spleenwort, *Asplenium montanum* Willd. × walking fern, *Camptosorus rhizophyllus* (L.) Link) from south of Shoals, Martin Co., Indiana (kindly provided by Warren P. Stoutamire) was opened at the same time spores of the

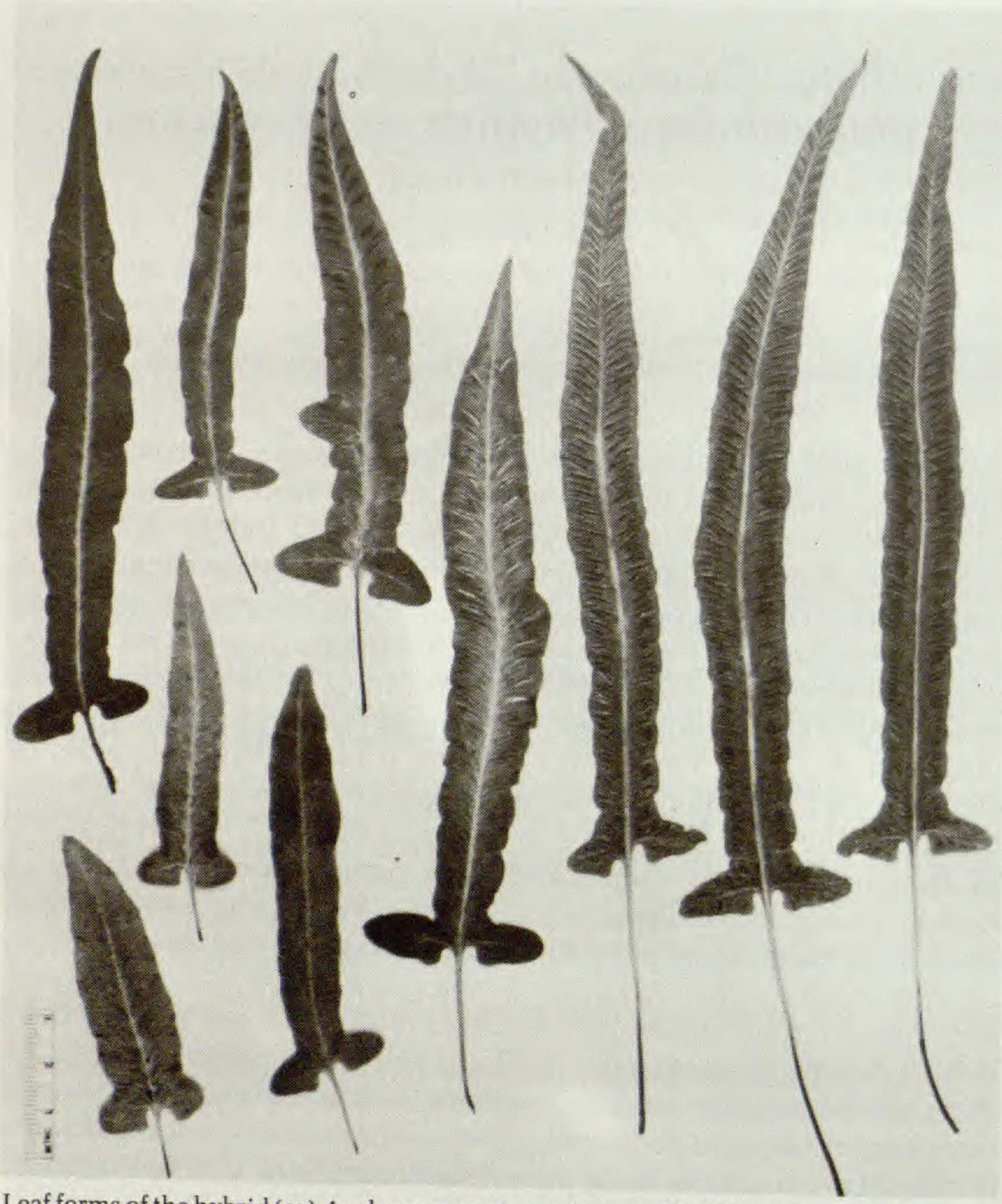

FIG. 1. Leaf forms of the hybrid (×) *Asplenosorus pinnatifidus* × *Phyllitis scolopendrium* var. *americana*. Note exaggerated basal auricles and near absence of lobation.

American hart's-tongue, *Phyllitis scolopendrium* var. *americana* Fern., were being sown in a nearby culture dish by Ethelda Hagenah. She later noticed a peculiar sporophyte among the (×)*A. pinnatifidus* and showed it to Wagner, who diagnosed it provisionally as a hybrid. The plant was vigorous and lived from 1965 to 1973, so we were able to obtain numerous fronds and make a few observations on its chromosomes. Had we tried to predict—assuming precise "in-the-middle" intermediacy (Barrington, 1986)—what this trihybrid of *Asplenium*, *Camptosorus*, and *Phyllitis* would look like, we would have erred for some characters. For example, by extrapolation, we would have expected it to have at least several pairs of lobes in the lower half of the blade, the scaliness of the stipe to be sparse, and the soral arrangement to be like that of *Asplenium* in half of the sori.

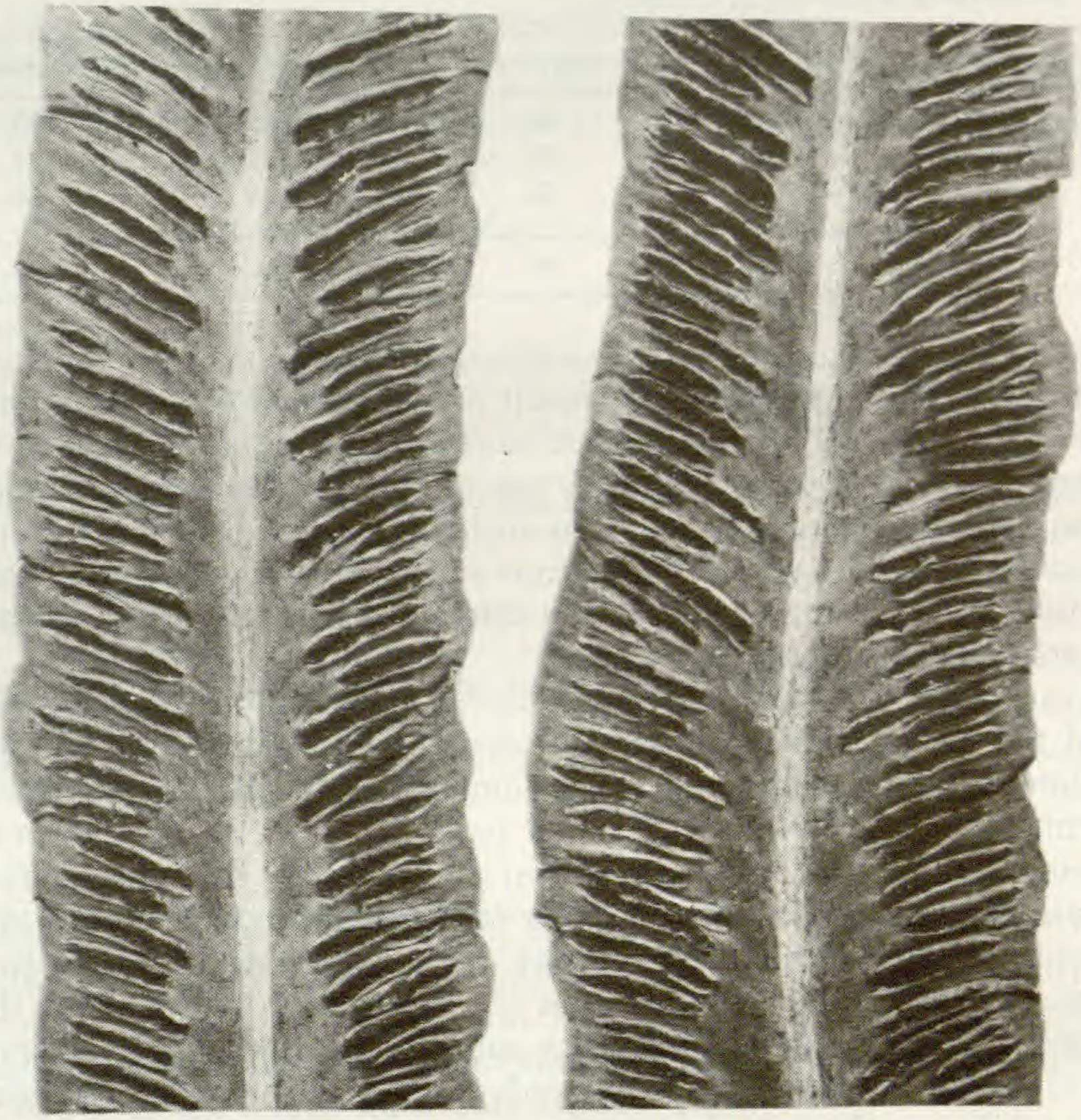

FIG. 2. Soriation of two full-sized fronds of the trihybrid, showing the close proximity of the sori and their almost uniformly double indusia.

The hybrid superficially resembles a coarse, thick-textured plant of *Camptosorus rhizophyllus*, with more or less crispate margins and enlarged basal auricles. The very shallow undulations of the margins are probably traces of (×)*A. pinnatifidus*. Only one out of approximately 50 fronds had a distinct lobe above the base (Fig. 1). We expected the hybrid to be moderately lobed above the base of the blade, because the plant contains genes of three genomes for simple leaves (two from *Phyllitis*, plus one from *Camptosorus* with one for twice compound leaves (*A. montanum*), but we did not expect it to be almost wholly without lobes. (P. Mick Richardson, in litt., had detected the existence of xanthones in our hybrid, confirming the presence of the *A. montanum* genome.) Why the basal auricles are so large and elongate is unexplained, for neither *Camptosorus* or *Phyllitis* has them so exaggerated. The sori are abundant and closer together than they are in *Phyllitis* (Fig. 2). Most curious is the fact that practically all of the sori have the characteristic doubly indusiate condition, due to the facing indusia from adjacent pairs of fertile veins, found in *Phyllitis* but not in either the *Asplenium* or the *Camptosorus*.

TABLE 1. Estimates of Chromosome Numbers.

Meiotic figure	Large pairs	Large singles	Small singles	# Chromosomes
A	15	40	73	143
B	18	34	76	146
C	14	52	70	150
Averages	15.7	42	73	146.4

The frond is thick, probably the result of the influence of the coriaceous textures of the *Phyllitis* and *Asplenium montanum* parents. Vein anastomoses are absent or rare. If present at all, only one or two are found, mainly along the costa near the base of the blade above the auricles. The petiole is short, and bears numerous reddish-brown hairs and narrow scales up to 4 mm long. The base of the petiole is the only portion that is darkly pigmented. Thus the petiole characters are close to those of *Phyllitis*.

The chromosomes of the hybrid at diakinesis were difficult to study because those of the *Phyllitis* genomes are decidedly larger than those of both the *Asplenium* and the *Camptosorus*, and a number of large pairs are formed. Large univalents may be confused with small pairs. None of the figures could be interpreted without difficulty, but the best three gave the estimates in Table 1. The estimated numbers are close to the expected 72 (*Phyllitis*) + 36 (*Asplenium*) + 36 (*Camptosorus*) = 144. The deviations of our estimated numbers are probably due to difficulties of assessing the figures rather than to aneuploidy: monosomics and trisomics and other aneuploid phenomena are apparently rare or absent among asplenioids in our experience. A certain amount of pairing between the homologous chromosomes of the tetraploid American *Phyllitis* is to be expected because it is probably an intraspecific polyploid derivative of the typical European diploid form.

DISCUSSION

The "trigeneric" hybrid described here is noteworthy because of the strongly differing features of the participating parents. Whether or not authors recognize them as belonging to separate genera, the elements are strongly divergent in several characters. The orthospecies, *Asplenium montanum*, a thick-textured plant of crevices in acidic rocks, the blades of which are 2–3-divided and the veins free, combined with the orthospecies *Camptosorus rhizophyllus*, a plant with leaves which are simple with a long "walking tip" to form the well known allotetraploid nothospecies, (×)*Asplenosorus pinnatifidus* (Wagner, 1954). *Phyllitis scolopendrium* var. *americana*, with its strap-shaped large simple fronds, remote "double sori," and tetraploid sets of chromosomes, combines with the foregoing to produce a curious conglomeration of character states. The simple, unlobed leaf structure derives from *Camptosorus* and *Phyllitis*, which dominate the frond structure to the extent that pinnation derived from *Asplenium montanum* is essentially eliminated. The net veins of *Camptosorus*

TABLE 2. Comparison of Two Different Trigeneric Hybrid Combinations.

	(×)*Asplenosorus pinnatifidus* (= *Asplenium montanum* × *Camptosorus rhizophyllus*, fertile form) × *Phyllitis scolopendrium* var. *americana*	(×)*Asplenosorus ebenoides* (= *Asplenium platyneuron* × *Camptosorus rhizophyllus*, fertile form) × *Phyllitis scolopendrium* var. *scolopendrium*
Texture	coriaceous	chartaceous
Lobation	no lobes above base (with rare exceptions)	commonly l-several lobes above base
Areoles	costal, only 1 (rarely 2), if present at all	costal, l-several near blade base
Soriation	*Phyllitis*-like, rarely otherwise. Medial, 1 mm apart	*Phyllitis*-like, occasionally otherwise. Inframedial, 1-4 mm apart
Petiole color	dark-pigmented, only at base	dark-pigmented, the color running into lower rachis
Petiole scales	numerous and dense, especially at petiole base	few and scattered
Chromosomes	ca. 144; 72 large ones, 72 small ones; ca. 15 pairs	108; 36 large ones, 72 small ones; no pairing

are obscured by inheritance from the other two parents, so that they are practically entirely free. Two unique features of *Phyllitis* play strong morphological roles in this hybrid: The sori are "double," and the petiole strongly scaly and short, thus maintaining these character states in spite of the other two genomes which show only "single" sori.

It is rewarding to compare the synthetic hybrid with another trigeneric combination, this one involving *Camptosorus rhizophyllus*, *A. platyneuron* (rather than *A. montanum*), and the diploid European variety of *Phyllitis scolopendrium* rather than the tetraploid American variety. This hybrid, which originated in the cultures of the late Kay Boydston at Fernwood, Michigan (Wagner, 1989), shows more lobing of the blade, as might be expected because of the lesser influence from *Phyllitis*, there being only one genome rather than two. Probably for the same reason there are fewer scales on the petiole, which is also longer. However, the "double-sorus" condition is retained as a dominant feature from *Phyllitis*. Also, the dark leaf axis of *Asplenium platyneuron* is surprisingly well developed in the hybrid, considering the pale axes from the other two genomes. A summary of the major differences between the two trigeneric hybrids is given in Table 2.

One of the prevalent problems in the study of fern nothospecies involves the relative expression of parental characters. Studies of various biochemical compounds, including such different entities as phenolic compounds and isoenzymes, demonstrate that the inheritance of these tend to be additive, the electrophoretic or chromatographic pattern of one species superposed upon that of the other. On the contrary, morphological characteristics, as is now well known, tend to be intermediate, i.e., somewhere between the extremes laid

down by the parents. Whether they show dominance of the phenotype of one parent over the other in any given trait may bear upon the interpretation on genetic controls of that morphological feature. Only rarely are all or most character states truly "medial" (Barrington's term for being precisely "in the middle" between the parents, Barrington, 1986).

While it is still too early to arrive at firm conclusions regarding this, it is tempting to speculate that experimental hybridization using parents with widely different morphologies may lead to possible insights concerning homologies, dominance effects, and morphogenesis. Continued work with synthesized hybrids may permit us to address questions like: Why is the "double sorus" of *Phyllitis* so strongly expressed in these hybrids? In contrast, why hasn't the reticulate venation of *Camptosorus* been more strongly expressed? For those especially interested in raising and culturing ferns as a hobby, it seems to us that artificially synthesizing new crosses may serve not only as an enjoyable and stimulating pastime, but may, in the end, produce something of scientific value.

Also, studies of hybrids like this one may bear on whether we recognize certain genera. Admittedly, although there is still much disagreement about this, the genera *Camptosorus* and *Phyllitis* may well be congeneric with *Asplenium*. Thus, whether we call the hybrids discussed here "intergeneric" or "intrageneric" depends on our taxonomic viewpoint.

Acknowledgments

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